STUDY OF SERUM ZINC LEVEL IN DIABETES MELLITUS AND ITS ROLE IN DEVELOPMENT OF COMPLICATIONS

Dissertation submitted in partial fulfillment of requirements for

M.D. DEGREE IN GENERAL MEDICINE
BRANCH I

Of

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY,
CHENNAI, INDIA.

MADRAS MEDICAL COLLEGE,
CHENNAI 600003

APRIL 2012

CERTIFICATE

This is to certify that the dissertation entitled "**STUDY OF SERUM ZINC LEVEL IN DIABETES MELLITUS AND ITS ROLE IN DEVELOPMENT OF COMPLICATIONS"** is a bonafide work done by **Dr.MAGESH.A.**, at Madras Medical College, Chennai in partial fulfillment of the university rules and regulations for award of M.D., Degree in General Medicine (Branch-I) during the academic year 2009 -2012.

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DECLARATION

I solemnly declare that this dissertation entitled "**STUDY OF SERUM ZINC LEVEL IN DIABETES MELLITUS AND ITS ROLE IN DEVELOPMENT OF COMPLICATIONS"** was done by me at Madras Medical College and Rajiv Gandhi Government General Hospital, during 2009-2012 under the guidance and supervision of**, Prof.K.SIVASUBRAMANIAN, M.D.** This dissertation is submitted to the Tamil Nadu Dr.M.G.R. Medical University towards the partial fulfillment of requirements for the award of M.D. Degree in General Medicine (Branch-I).

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ACKNOWLEDGEMENT

At the outset, I thank **Prof.V.KANAGASABAI M.D.,** Dean, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai-3 for having permitted me to use hospital data for the study.

I am very much thankful to **Prof.V.PALANI M.S.,** Medical Superintendent, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai-3 for permitting me to carry out my study.

I am grateful to **Prof.C.RAJENDIRAN, M.D.,** Director and Professor, Institute of Internal Medicine, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai-3.

I am indebted to **Prof.K.SIVASUBRAMANIAN, M.D.,** Professor of Medicine, Institute of Internal Medicine, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai-3 for his valuable guidance.

I am indebted to **Prof.C.R.ANAND MOSES,M.D**.,Professor and HOD Of Diabetology,Madras Medical college and Rajiv Gandhi Government General Hospital,Chennai-3 for his cooperation and guidance.

I would like to thank **Dr.A.MURUGESAN,M.D., Dr.G.RAJAN,M.D.,Dr.S.GOPALAKRISHNAN,M.D.,**Assistant Professors, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai-3 for their scrutiny.

I would also like to thank all the professors and assistant professors of the Department of Cardiology for their continuous support and expert guidance.

I express my sincere gratitude to all the patients who participated in the study. Lastly,I thank all my professional colleagues for their support and valuable criticism.

ABBREVIATIONS

ADA	:	American Diabetes Association
AGE	:	Advanced Glycosylation End products
BMI	:	Body Mass Index
CAD	:	Coronary Artery Disease
Cu	:	Copper
CVA	:	Cerebro Vascular Accident
DCCT	:	Diabetes Control and Complication Trial
DNA	:	DeoxyriboNucleic Acid
DAG	:	Diacyl Glycerol
DM	:	Diabetes Mellitus
ECM	:	Extra Celllular Matrix
FBS	:	Fasting Blood Sugar
GLUT	:	Glucose Uptake and Transport

HBA1C	:	Glycosylated Haemoglobin
HNF	:	Hepatocyte nuclear transcription factor
IL	:	Interleukin
MDA	:	Malondialdehyde
MODY	:	Maturity onset diabetes of Young
NCS	:	Nerve conduction study
PAD	:	Peripheral Artery Disease
RDA	:	Recommended daily allowance
SOD	:	Superoxide dismutase
STZ	:	Streptozocin
TNF	:	Tumor necrosis factor
Zn	:	Zinc

CONTENTS

INTRODUCTION

Diabetes mellitus (DM) is characterised by hyperglycaemia due to disturbances in the metabolism of carbohydrate, fat and protein because of abnormalities in the availability of insulin or insulin action (65).

Currently number of diabetic patients in worldwide is estimated to be 150 millions, two third of which are residing in developing countries. The number is predicted to double by 2025, with the greatest number of cases in India and China alone.

Even though diabetes mellitus is an endocrine disease in origin, its major manifestations are those of a metabolic disease. The characteristic symptoms are excessive thirst, polyuria, pruritus, and otherwise unexplained weight loss. The secondary complications of diabetes is due to the thickening of basement membrane.

The most dominant feature of the metabolism in diabetes mellitus is an abnormally high concentration of blood glucose. This can be either due to an abnormally high rate of glucose production or of impaired glucose utilisation. It is now accepted that the high

blood glucose level is the result of combination of both of these processes.

The secondary complications seen in diabetic patients are found to be due to alterations in vascular basement membrane composition as well as accumulation of glucose derived reaction products owing to over utilisation of glucose in insulin independent tissues [2].

Various authors have shown that hyperglycaemia leads to an increase in serum glycated proteins [3, 4, 5] along with alterations in other atherogenic risk factors. Disturbances in mineral metabolism are also noticed [6] and it is not known whether differences in trace element status are a consequence to the expression of the disease.

Zn is an essential trace mineral directly involved in the physiology and action of insulin. Insulin is stored as Zn crystals in the β cells of the pancreas. It has been suggested that abnormal Zn metabolism may play a role in the pathogenesis of diabetes and some of its complications [27]. Zn depletion has several potential clinical implications. It is speculated that Zn repletion could improve insulin sensitivity in patients with DM and reduce the

severity of certain complications of this disease [28]. In order to understand the underlying patho biochemical inter-relationships of the late complications of diabetics in more detail, this study was undertaken.

AIMS & OBJECTIVES

- To detect serum zinc level in patients with diabetes mellitus.
- To compare the serum zinc level in newly diagnosed diabetic patients & in those with complications.
- To find out the relationship between zinc deficiency & complications of diabetes.

REVIEW OF LITERATURE

ZINC

Zinc is the 24th most abundant element in the Earth's crust and has five stable isotopes. The element was probably named by the alchemist Paracelsus after the German word *zinke*. German chemist Andreas Sigismund Marggraf is normally given credit for discovering pure metallic zinc in 1746. Work by Luigi Galvani and Alessandro Volta uncovered the electrochemical properties of zinc by 1800. A variety of zinc compounds are commonly used, such as zinc carbonate and zinc gluconate (as dietary supplements), zinc chloride (in deodorants), zinc pyrithione (anti-dandruff shampoos), zinc sulfide (in luminescent paints), and zinc methyl or zinc diethyl in the organic laboratory.

BIOCHEMICAL FUNCTIONS OF ZINC

Zinc is an essential trace element, necessary for plants[29] animals and microorganisms. Zinc is found in nearly 100 specific enzymes, serves as structural ions in transcription factors and is stored and transferred in metallothioneins. It is "typically the second most abundant transition metal in organisms"

after iron and it is the only metal which appears in all enzyme classes [29].

There are 2–4 grams of zinc distributed throughout the human body. Most zinc is in the brain, muscle, bones, kidney, and liver, with the highest concentrations in the prostate and parts of the eye. Semen is particularly rich in zinc, which is a key factor for prostate gland function and reproductive organ growth.

Zinc plays ubiquitous biological roles in the prostate [34]. It interacts with a wide range of organic ligands[34] and has roles in the metabolism of RNA and DNA, signal transduction, and gene expression. It also regulates apoptosis.

A 2006 study estimated that about 10% of human proteins (2800) potentially bind zinc, in addition to hundreds which transport and traffic zinc; similar to the Silico study in the plant *Arabidopsis thaliana* which found 2367 zinc-related proteins[29].

In the brain, zinc is stored in specific synaptic vesicles by glutaminergic neurons [35] and can modulate brain excitability[34]. It plays a key role in synaptic plasticity and so in learning. However it has been called "the brain's dark horse"[39] since it also can be a neurotoxin, suggesting that

zinc homeostasis plays a critical role in normal functioning of the brain and central nervous system.

Zinc is an efficient lewis acid, making it a useful catalytic agent in hydroxylation and other enzymatic reactions. The metal also has a flexible coordination geometry, which allows proteins using it to rapidly shift conformations to perform biological reactions. Two examples of zinc-containing enzymes are carbonic anhydrase and carboxy peptidase, which are vital to the processes of carbon dioxide (CO_2) regulation and digestion of proteins, respectively.

In vertebrate blood, carbonic anhydrase converts CO_2 into bicarbonate and the same enzyme transforms the bicarbonate back into CO_2 for exhalation through the lungs. Without this enzyme, this conversion would occur about one million times slower at the normal blood pH of 7 or would require a pH of 10 or more. Carboxypeptidase cleaves peptide linkages during digestion of proteins. A coordinate covalent bond is formed between the terminal peptide and a C=O group attached to zinc, which gives the carbon a positive charge. This helps to create a hydrophobic pocket on the enzyme near the zinc, which attracts the non-polar part of

the protein being digested [39].Zinc also serves a purely structural role in zinc fingers twists and clusters[40].

METABOLISM OF ZINC

In blood plasma, zinc is bound to and transported by albumin (60%, low-affinity) and transferrin (10%). Since transferrin also transports iron, excessive iron reduces zinc absorption, and vice-versa. A similar reaction occurs with copper. The concentration of zinc in blood plasma stays relatively constant regardless of zinc intake. Cells in the salivary gland, prostate, immune system and intestine use zinc signaling as a way to communicate with other cells [42].

Zinc may be held in metallothionein reserves within microorganisms or in the intestines or liver of animals. Metallothionein in intestinal cells is capable of adjusting absorption of zinc by 15–40%. However, inadequate or excessive zinc intake can be harmful; excess zinc particularly impairs copper absorption because metallothionein absorbs both metals.

ZINC DEFICIENCY

Hypozincemia is a condition where insufficient zinc is available for metabolic needs.

Prevalence

In fact, one-third of the world population is at risk of zinc deficiency, ranging from 4 to 73% depending on the country. Zinc deficiency is the fifth leading risk factor for disease in the developing world. Providing micronutrients, including zinc, to humans is one of the four quick-win solutions to major global problems identified in the Copenhagen consensus from an international panel of distinguished economists. Conservative estimates suggest that 25% of the world's population is at risk for zinc deficiency[44]

Causes

Hypozincemia is usually a nutritional deficiency, but can also be associated with malabsorption, diarrhoea, acrodermatitis enteropathica, chronic liver disease, chronic renal disease, sickle cell diasease, diabetes, malignancy, and other chronic illnesses [45]. It can also occur after bariatric surgery.

Zinc deficiency is typically the result of inadequate dietary intake of zinc, disease states that promote zinc losses, or physiological states that require increased zinc. Populations that consume primarily plant based diets that are low in bioavailable zinc often have zinc deficiencies [46] [47]. Diseases or conditions

that involve intestinal absorption promote zinc losses. Fecal losses of zinc caused by diarrhea is one contributing factor [55], often common in developing countries. Changes in intestinal tract absorbability and permeability due, in part, to viral, protozoal, and bacteria pathogens may also encourage fecal losses of zinc[49].Physiological states that require increased zinc include periods of growth in infants and children as well as in mothers during pregnancy[50].

SIGNS AND SYMPTOMS

Signs of zinc deficiency include hair loss,skin lesions, diarrhea, and wasting of body tissues. A lack of zinc can contribute to acne [51]. Vision[52][53], taste ,smell and memory are also connected with zinc. A deficiency in zinc can cause malfunctions of these organs and functions.

One easily recognized sign which may be caused by zinc deficiency is white spots, bands, or lines on fingernails (leuconychia).An occasional white spot is an evidence that the immune system has overcome a bacterial or some other systemic infection. Some women may have multiple parallel white bands or lines on the fingernails marking menstrual cycles when marginal zinc deficiency was present.

Cognitive and motor function impairment

Cognitive and motor function may also be impaired in zinc deficient children. Zinc deficiency can interfere with many organ systems especially when it occurs during a time of rapid growth and development, when nutritional needs are high, such as during infancy [57][43]. Low maternal zinc status has been associated with less attention during the neonatal period and worse motor functioning[59]. In some studies, supplementation has been associated with motor development in very low birth weight infants and more vigorous and functional activity in infants and toddlers[59].

Diarrhoea and pneumonia

Zinc deficiency contributes to an increased incidence and severity of diarrhoea and pneumonia[48]. Studies have shown that zinc treatment results in 25 percent reduction in duration of acute diarrhoea and a 40 percent reduction in treatment failure or death in persistent diarrhoea[60]. Studies have found that a ten-day therapy of zinc treatment can considerably reduce the duration and severity of diarrheal episodes, decrease stool output, and lessen the need for hospitalization. Zinc may also prevent future diarrhoea episodes for up to three months. A zinc taste test which finds out the taste

disturbances in an individual may have potential for diagnosing its deficiency [54].

Dysmenorrhoea

High dose of zinc, 30 mg 1-3 times a day, prevents dysmenorrhoea[62] .

Pregnancy

Zinc deficiency during pregnancy can negatively affect both the mother and fetus. A review of pregnancy outcomes in women with acrodermatitis enteropathica, reported that out of every seven pregnancies, there was one abortion and two malfunctions, suggesting the human fetus is susceptible to the teratogenic effects of severe zinc deficiency. However, a review on zinc supplementation trials during pregnancy did not report a significant effect of zinc supplementation on neonatal survival.

Effect of zinc on lymphoid and myeloid cells

Thymulin, a thymic hormone, requires zinc for its activity[37][33]. In zinc deficiency, Th_1 cytokines decrease, but Th_2 cytokines are not affected. Thus, Th_1 shifts to Th_2 function. Zinc decreases the gene expression and generation of TNF α, IL-1β,IL- 8 cytokines.

Treatment of zinc defeciency

Zinc supplementation has been shown to reduce diarrhea prevalence and mortality in children younger than 5 years of age [64]. To combat zinc deficiency, five intervention strategies can be used:

1) Supplementation using medicines

2) Food fortification through the incorporation of zinc additives in food

3) Dietary modification/diversification

4) Genetic biofortification through plant breeding

5) Agronomic biofortification through zinc fertilization.

EFFECTS OF DIABETES ON ZINC METABOLISM

Hypozincemia may be the result of hyperzincuria or decreased gastrointestinal absorption of Zn or both in diabetes. It appears the hyperzincuria, is a result more of hyperglycemia than of any specific effect of endogenous or exogenous insulin on the renal tubule. Isbir et al demonstrated a 20% decrease in serum Zn ($p < 0.01$) in Type I diabetes, apparently the result of hyperzincuria [7].

El Yazigi et al evaluated both Type I and II diabetics and found that the absolute and creatinine corrected urinary excretions were greater in diabetics than in matched controls and found a positive correlation between Zn excretion and hemoglobin Alc concentrations [8].

In Type II Diabetes,insulin reduces the hyperzincuria while other agents had no effect on zinc excretion[9].

It has also been postulated that hyperglycemia interferes with the active transport of Zn back into the renal tubular cells. Administration of insulin reduces but does not appear to completely ameliorate the hyperzincuria [11][12].

McNair et al [13] confirmed that hyperzincuria occurs in relationship to the degree of hyperglycemia, but not glycosuria. Kinlaw et al [10] demonstrated abnormal Zn tolerance tests in diabetic patients suggestive of decreased absorption.

Escobar also demonstrated a down regulation of fractional Zn transport in the gut and kidney which may be related to increased production of metallothionein in diabetics. Metallothionein is an intracellular cation binding protein which appears to act as an inhibitor of Zn transport. This decrease in gastrointestinal

absorption, coupled with hyperzincuria, could account for significant loss of intracellular Zn [14][13].

While it is clear that urinary excretion of Zn is markedly increased in individuals with diabetes, if hyperglycemia is the primary etiology, replacement with oral Zn supplementation should provide sufficient treatment[38].

EFFECTS OF ZINC ON DIABETES MELLITUS (PRIMARY DISEASE EFFECTS)

In 1966, Quarterman et al[15] demonstrated that diet induced zinc deficiency in rats resulted in a decrease in the ability of the pancreas to secrete insulin in response to a glucose load—a hallmark of diabetes.

A few years later, Boquist et al [16] demonstrated a decrease in glucose tolerance with no change in insulin production in zinc deficient hamsters in response to an iv glucose load. They also demonstrated decreased granulation of the islet cell with both light and electron microscopy in zinc deficiency [16]. Since zinc is intrinsic to the storage granulization of insulin within the beta cell and increased insulin secretion reduces beta cell zinc concentration, these data are compatible with decreased islet cell insulin content in zinc deficiency states [17].

Because zinc is a necessary factor in a variety of "antioxidant" enzymes, particularly superoxide dismutase, catalase and peroxidase, alterations of zinc metabolism such that adequate zinc is unavailable for these enzymes might be expected to contribute to the tissue damage observed in diabetes [18].

Rabinovitch et al [19] examined the relationship between cytokine induced (interleukin 1b, tumor necrotic factor (TNF) and interferon gamma) pancreatic beta cell destruction, production of malondialdehyde (MDA), an end product of lipid peroxidation, and nitrite, the end product of nitric oxide. These studies suggested that cytokines are toxic to the human beta cell by producing oxygen free radicals, lipid peroxidation, and aldehyde production in the islets and that MDA was one of the cytotoxic mediators [18][19].

Other investigators have suggested that the Zn-metallothionein complex in the islet cell provides protection against free radicals produced in the cell from any cause, and certainly the immune mediated cytokine provoked oxidative stress would be a significant oxidative stress. The more depleted the intracellular Zn stores, the less able the cell is to defend itself against this oxidative load[30]. This provides a potential mechanism for zinc deficiency to affect the progress of Type I diabetes.

Zimny et al have demonstrated induction of metallothionein production in the islet cell in response to Streptozocin (STZ) induced OH radical production [20]. Yang and Cherian [21] demonstrated STZ-induced lipid peroxidation and decreased superoxide dismutase (SOD). Roza showed marked decreases in pancreatic SOD and catalase antioxidant activity which preceded the loss of beta cell function suggesting increased beta cell vulnerability to free radical attack and cell destruction in genetically diabetic rats [22]. All of these data suggest a role for Zn in the protection of the beta cell against the immune-mediated free radical attack.

In Type II diabetes, there is no good evidence for oxidative stress as a major factor in the development of either insulin deficiency or islet cell damage, but there is clear evidence for increased secretion of insulin, at least early in the progress of the disease. Since Zn leaves the cell with insulin, the greater secretion of insulin causes cellular depletion of Zn. The cell can make more insulin, but it cannot make more Zn and, with hyperzincuria, the Zn co-secreted is more likely to be excreted and not available for re-uptake into the cellular pool. With the slow loss of intracellular zinc, the less insulin is secreted for a given glucose level and the

islet cell becomes more vulnerable to all sorts of damage. This matches the clinical picture in which, after prolonged hyperglycemia and inability of the islet cell to make enough insulin to control the glucose, there is a loss of islet cell altogether. This provides a mechanism by which Zn deficiency may affect the progress of Type II diabetes [23].

EFFECTS OF ZINC ON DIABETES MELLITUS (SECONDARY COMPLICATIONS)

It has become clear in recent years that hyperglycemia is a major culprit in the development of the microvascular complications which include retinopathy, nephropathy, neuropathy and the small vessel occlusions as well as birth defects including fetal malformations and macrosomia. It also appears clear that, while hyperglycemia is a major contributor, it is not the only contributor to the development of these complications.

In mice made diabetic with STZ there was a marked increase in fetal loss and malformation as compared with nondiabetic mice. In similar, but transgenic mice for the human copper-zinc superoxide dismutase (Cu-Zn SOD), there were fewer fetal malformations than in controls without the gene suggesting SOD reduces diabetic embryopathy, presumably by reducing oxygen free

radicals [24]. In other animal studies Minami et al [25] demonstrated an increase in the progression of diabetic nephropathy in STZ diabetic rats when Zn deficiency was induced either by increased renal excretion or by dietary induced deficiency.

In humans, Faure demonstrated some protective effect of Zn supplementation for the development of diabetic retinopathy associated with an increase in SOD. This suggested that the observed decrease in retinopathy may be the result of decreased lipid peroxidation of the retinal polyunsaturated fatty acids [26].

DIABETES MELLITUS

DEFINITION

The term diabetes mellitus describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action[65], or both. The effects of diabetes mellitus include long–term damage, dysfunction and failure of various organs.

SYMPTOMS AND SIGNS

Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. In its most severe forms, ketoacidosis or a non ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death. Often symptoms are not severe, or may be absent, and consequently hyperglycaemia sufficient to cause pathological and functional changes may be present for a long time before the diagnosis is made. The long–term effects of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, and/or neuropathy with risk of foot ulcers,

amputation, Charcot joints, and features of autonomic dysfunction, including sexual dysfunction.

PATHOGENESIS OF DIABETES

Several pathogenetic processes are involved in the development of diabetes. These include processes which destroy the beta cells of the pancreas with consequent insulin deficiency, and others that result in resistance to insulin action. The abnormalities of carbohydrate, fat and protein metabolism are due to deficient action of insulin on target tissues resulting from insensitivity or lack of insulin. The aetiological types designate defects, disorders or processes which often result in diabetes mellitus.

TYPE 1

Type 1 indicates the processes of beta–cell destruction [65] that may ultimately lead to diabetes mellitus in which insulin is required for survival to prevent the development of ketoacidosis, coma and death. An individual with a Type 1 process may be metabolically normal before the disease is clinically manifest, but the process of beta–cell destruction can be detected.

Type 1 is usually characterized by the presence of anti–GAD, islet cell or insulin antibodies [67] which identify the autoimmune processes that lead to beta–cell destruction. In some subjects with

this clinical form of diabetes, particularly non–Caucasians, no evidence of an autoimmune disorder is demonstrable and these are classified as “Type 1 idiopathic”.

TYPE 2

It is due to predominant insulin resistance with relative insulin deficiency or predominant insulin secretory defect with/without insulin resistance[68]. Diabetes mellitus of this type was previously encompassed as non–insulin–dependent diabetes, or adult–onset diabetes. It is a term used for individuals who have relative (rather than absolute) insulin deficiency.

People with this type of diabetes frequently are resistant to the action of insulin. At least initially, and often throughout their lifetime, these individuals do not need insulin treatment to survive. This form of diabetes is frequently undiagnosed for many years because the hyperglycaemia is often not severe enough to provoke noticeable symptoms of diabetes. Nevertheless, such patients are at increased risk of developing macro vascular and micro vascular complications [69].

The majority of patients with this form of diabetes are obese, and obesity itself causes or aggravates insulin resistance [70][66].

Many of those who are not obese by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region. Ketoacidosis is infrequent in this type of diabetes; when seen it usually arises in association with the stress of other illness such as infection .

The risk of developing Type 2 diabetes increases with age, obesity, and lack of physical activity . It occurs more frequently in women with prior GDM and in individuals with hypertension or dyslipidaemia .Some patients who present with a clinical picture consistent with Type 2 diabetes have auto antibodies similar to those found in Type 1 diabetes, and may masquerade as Type 2 diabetes if antibody determinations are not made. Patients who are non–obese or who have relatives with Type 1 diabetes and who are of Northern European origin may be suspected of having late onset Type 1 diabetes.

COMPLICATIONS OF DIABETES MELLITUS

Acute complications

Diabetic ketoacidosis

Diabetic nonketotic, hyperosmolar coma

Hypoglycemia

CHRONIC COMPLICATIONS

Microvascular

Retinopathy

Nephropathy

Neuropathy

Macrovascular

Cerebrovascular

Cardiovascular

Peripheral vascular disease

MICROVASCULAR COMPLICATIONS

Microvascular complications are specific to diabetes and do not occur without longstanding hyperglycaemia. Other metabolic, environmental and genetic factors are undoubtedly involved in their pathogenesis.

Both T1DM and T2DM are susceptible to microvascular complications, although patients with T2DM are older at presentation and may die of macrovascular disease before microvascular disease is advanced.

The duration of diabetes and the quality of diabetic control are important determinants of microvascular disease but, because of other individual factors, do not necessarily predict their developent in individual patients.Different microvascular complications are commonly associated in individual patients, but their prevalence as a function of the duration or severity of diabetes may differ markedly.

Background retinopathy is rare before 5 years of diabetes but its prevalence increases steadily thereafter to affect over 90% of patients after 20 years. After several years of diabetes, the risk of proliferative changes is about 3% of patients per year, with a cumulative total of over 60% after 40 years.

Diabetic nephropathy affects 20-40% of patients with T1DM, particularly those presenting before puberty and possibly those with an inherited tendency to hypertension. T2DM patients are also susceptible to nephropathy.

Over 40% of subjects with T1DM survive more than 40 years, half of them without developing significant micro-vascular complications.

PATHOPHYSIOLOGY OF MICROVASCULAR DISEASE

In diabetes, the microvasculature shows both functional and structural abnormalities. The structural hallmark of diabetic microangiopathy is thickening of the capillary basement membrane. The main functional abnormalities include increased capillary permeability, blood flow and viscosity, and disturbed platelet function. These changes occur early in the course of diabetes and precede organ failure by many years.

Many chemical changes in basement membrane composition have been identified in diabetes, including increased type IV collagen and its glycosylation products. In patients with poorly controlled diabetes, even of short duration, blood flow is increased in many tissues including skin, retina and kidney. In the latter this is reflected by an elevated glomerular filtration rate. Increased capillary permeability is manifested in the retina by leakage of fluorescein and in the kidney by increased urinary losses of albumin which predict eventual renal failure. Both defects probably reflect a generalized vascular abnormality which may also involve the intima of the large vessels.

Platelets from diabetic patients show an exaggerated tendency to aggregate, perhaps mediated by altered prostaglandin

metabolism[71] . Plasma and whole blood viscosity are increased whereas red blood cell deformability is decreased in diabetes.

These defects together with the platelet abnormalities may cause stasis in the microvasculature, leading to increased intravascular pressure and to tissue hypoxia. The production by endothelial cells of von Willebrand factor and endothelial derived relaxing factors (mainly nitric oxide) may also be abnormal in diabetes and could contribute to tissue damage.

BIOCHEMICAL BASIS OF MICROVASCULAR DISEASE

Prolonged exposure to elevated glucose concentrations damages tissues by causing either acute, reversible metabolic changes (mostly related to increased polyol pathway activity and glycosylation of proteins) or cumulative irreversible changes in long lived molecules (formation of advanced glycosylation end products /AGE/ on matrix proteins such as collagen and on nucleic acids and nucleoproteins). In insulin independent tissues (nerve, lens, retina) hyperglycaemia causes elevated tissue glucose levels.

The enzyme aldose reductase catalyses the reduction of glucose to its polyol, sorbitol, which is subsequently converted to fructose. Sorbitol does not easily cross cell membranes and its

accumulation may cause damage by osmotic effect (e.g. in the lens). In addition, increased sorbitol production is partly responsible for tissue depletion of myoinositol, a molecule structurally related to glucose. Hypergylcaemia itself inhibits myoinositol uptake into cells. Animal studies indicate that tissue myoinositol depletion may cause abnormalities on peripheral nerve function.

ADVANCED GLYCOSYLATION END PRODUCTS

In long lived molecules early glycosylation products slowly and irreversibly form complex cross-linkings termed advanced glycosylation end products (AGE). Pathological consequences of AGE cross linking include covalent binding of proteins (e.g. LDL, albumin and IgG) to vessel walls; crosslinking of matrix components in vessel walls causing resistance to enzymatic degradation; and disturbed three-dimensional structure and altered binding of anionic proteoglycans which influence charge on the vessel wall and its interaction with blood-borne protein. Monocyte-macrophages have a high affinity receptor for AGE and binding of AGE may release cytokines(TNF, IL-1) and inflammatory reactions inside the vessel wall followed by atherosclerotic process.

PATHOGENESIS OF DIABETIC RETINOPATHY

Histologically the earliest lesion is thickening of the capillary basement membrane. On fluorescein angiography the first abnormality is the capillary dilatation. Localised capillary dilatation is called microaneurysm. Microaneurysm may give rise to haemorrhage or exudate. Vascular occlusion, initially of capillaries and later of arteries and veins, leads to nonperfusion areas of retina. Large ischaemic areas are the stimulus of new vessel formation[1].

STAGES OF DIABETIC RETINOPATHY

Background retinopathy

Capillary dilatation (later with leakage)

Capillary occlusion

Microaneurysms

Blot haemorrhages and lipid rich hard exudates.

Preproliferative retinopathy

Cotton-wool spots (retinal ischemia)

Venous abnormalities (loops, beading and reduplication)

Arterial abnormalities (variation of caliber, narrowing of segments)

Intraretinal microvascular abnormalities (clusters of dilated abnormal capillaries lying within the retina).

Proliferative retinopathy

New vessels arise in the periphery and/or on the optic disc, eventually with a fibrous tissue covering. The visual complications are caused by vitreous retraction which leads to haemorrhage and to traction and detachment of the retina.

Maculopathy

Heralded by rings of hard exudates approaching the fovea. Maculopathy occurs mostly in T2DM and can cause severe visual loss.

Thrombotic glaucoma is due to new vessels and fibrous tissue proliferating in the angle of the anterior chamber, preventing drainage of the aqueous. It is associated with rubeosis iridis (neovascularisation of the iris) and causes severe pain and irreversible blindness

DIABETIC NEPHROPATHY

Albuminuria in diabetic nephropathy is due to glomerular capillary damage and reflects generalised damage to the microcirculation and large vessels, too. Nephropathic patients have

an increased incidence of retinopathy and a ten-fold increase in cardiovascular mortality which is the major cause of death in nephropathic T2DM patients.

Diabetic nephropathy is defined by persistent albuminuria (>300 mg/day), declining glomerular filtration rate and rising blood pressure. Established nephropathy follows several years of incipient nephropathy, characterised by microalbuminuria (30 – 300 mg/day).

In T1DM, nephropathy develops in about 35% of cases, especially in males and in those whose diabetes presents before the age of 15 years. The incidence of nephropathy peaks after 15-16 years of diabetes and declines thereafter. In T2DM prevalence of nephropathy about 10%.

The prevention of diabetic nephropathy: excellent diabetes control, agressive antihypertensive treatment, ACE inhibition (without hypertension,too)

METHODS & METHODOLOGY

Study centre : Rajiv Gandhi Government General Hospital, Chennai – 3

Study duration : 6 months

Study design : Case control study.

Sample size : 100 patients ; 50 controls.

INCLUSION CRITERIA

Patients with newly diagnosed Diabetes mellitus attending Diabetology OPD and those who are admitted to the medical wards with complications of Diabetes Mellitus.

EXCLUSION CRITERIA

Infections: Tuberculosis,HIV.

Malignancy

Cirrhosis

Inflammatory bowel disease

Patient on ATT, OCP, Valproate, Penicillamine, Iron supplements

Pregnancy

Sickle cell disease

DATA COLLECTION METHODS :

Collection of data as per proforma with consent from patients with diabetes mellitus in the Institute of Internal Medicine wards & in diabetology OP

SELECTION CRITERIA

Diabetes

Diagnostic criteria[32].

- Symptoms of diabetes mellitus plus random blood glucose concentration 11.1mmol/l (200mg/dl) or
- Fasting plasma glucose 7mmol/l (126mg/dl) or
- HbA1c >6.5% or
- 2 –hour plasma glucose 11.1mmol/l (200mg/dl) during an oral glucose tolerance test.

Random is defined as without regard to time since the last meal. Fasting is defined as no caloric intake for at least 8 h. The test should be performed in laboratory certified according to A1C standards of the Diabetes Control and Complications Trial. The test should be performed using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water, not recommended for routine clinical use. In the absence of unequivocal hyperglycemia

and acute metabolic decompensation, these criteria should be confirmed by repeat testing on a different day.

SYSTEMIC HYPERTENSION

Patients with Systolic BP > 130 & Diastolic BP > 80 mmHg

OBESITY

Patients with BMI > 25 Kg/sq.m.

DYSLIPIDEMIA

Total Cholesterol > 240 mg/dl

LDL – Cholesterol > 130 mg/dl

HDL – Cholesterol < 40 mg/dl(males) ; < 50 mg/dl (females)

Triglycerides > 150 mg/dl

MICROVASCULAR COMPLICATIONS

Retinopathy – Retinal changes in fundus examination.

Nephropathy – Presence of Microalbuminuria in urine.

Neuropathy – Symptoms ,Signs & NCS Abnormalities.

MACROVASCULAR COMPLICATIONS

Coronary heart disease – History, ECG, TMT & ECHO Abnormalities.

Peripheral vascular disease – Examination & Doppler Study.

Cerebro vascular accident – History, Examination & Imaging Study.

STATISTICAL ANALYSIS

Following statistical methods have been employed in the present study.

Independent samples 't' test-Unpaired.

Independent samples 't' test-Paired.

One-way Analysis of Variance (ANOVA).

SPSS Version 13

EXCEL 2010

The study was approved by ethics committee of the hospital.

ZINC ESTIMATION:[58,66]

Method – calorimetric method:

Principle: Zinc is an alkaline medium reacts with nitro-paps to form a purple coloured complex.Intensity of the complex formed is directly proportional to the amount of zinc present in the sample.

Zinc + nitro-paps ⟹ purple colour complex

NORMAL REFERENCE VALUES

serum-60-120micro gram/dl.

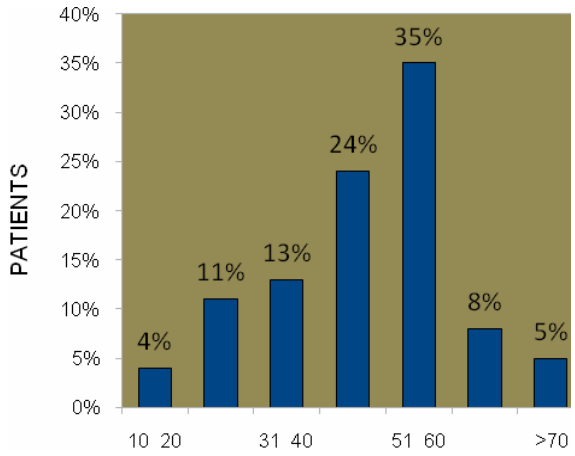
urine-100-1000micro gram/24 hrs.

OBSERVATION AND RESULTS

Table 1: Age incidence

Age(years)	No.of patients	%
10 to 20	4	4
21 to 30	11	11
31 to 40	13	13
41 to 50	24	24
51 to 60	35	35
61 to 70	8	8
>70	5	5

Chart showing age incidence in years:

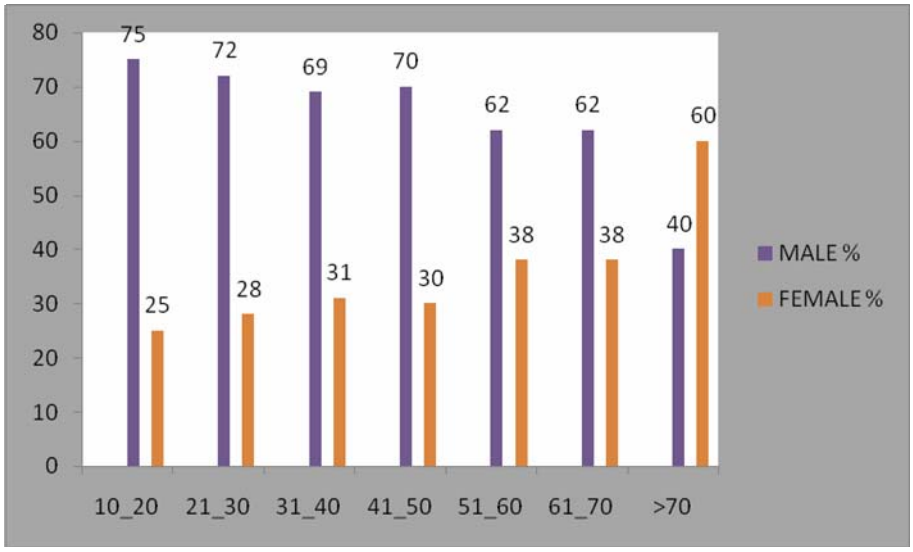


In the study group of 100 patients with Diabetes,35% were seen in the age group of 51-60 years,24% were seen in the age group of 41-50 years.Maximum incidence-35% was seen in the age group of 51-60 years.

Table 2: Age and sex distribution

Age	Total		Male		Female	
	No.	%	No.	%	No.	%
10 to 20	4	4	3	75	1	25
21 to 30	11	11	8	72	3	28
31 to 40	13	13	9	69	4	31
41to 50	24	24	17	70	7	30
51 to 60	35	35	22	62	13	38
61to 70	8	8	5	62	3	38
>70	5	5	2	40	3	60

Chart showing age and sex distribution

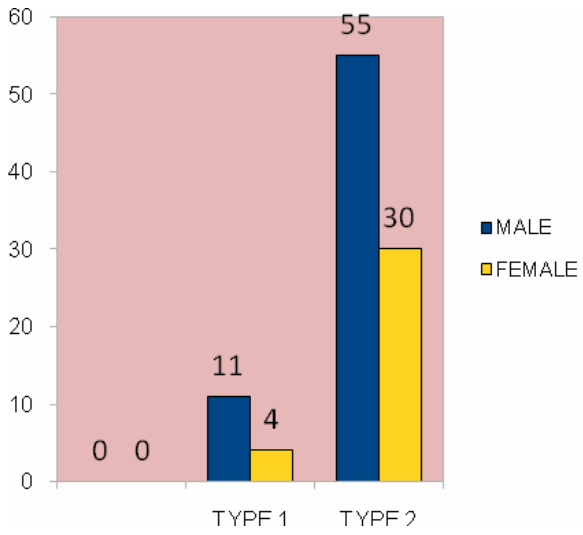


In the study group of 100 patients with DM, 66% were males, 34% were females. Male :female ratio-1.9:1.

Table: 3 Type of diabetes

	Total	**Male**		**Female**	
	No.	No.	%	No.	%
Type 1	15	11	73	4	27
Type 2	85	55	65	30	35

Chart showing the distribution of type of diabetes:

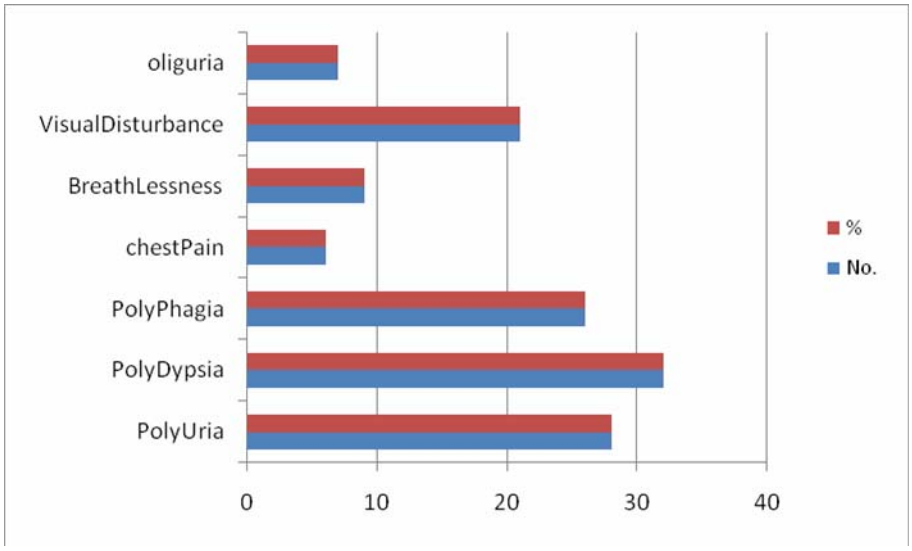


In the study group of 100 patients with DM,85% belonged to Type 2 DM,of which 65% were males,35% females.Patients with Type 1 DM formed 15%,of which 73% were males,27% were females.

Table: 4 Symptomatology

Symptomatology	Total	%
Polyuria	28	28
Polydipsia	32	32
Polyphagia	26	26
Chestpain	6	6
Breathlessness	9	9
Visualdisturbance	21	21
Oliguria	7	7

Chart showing the various symptomatology

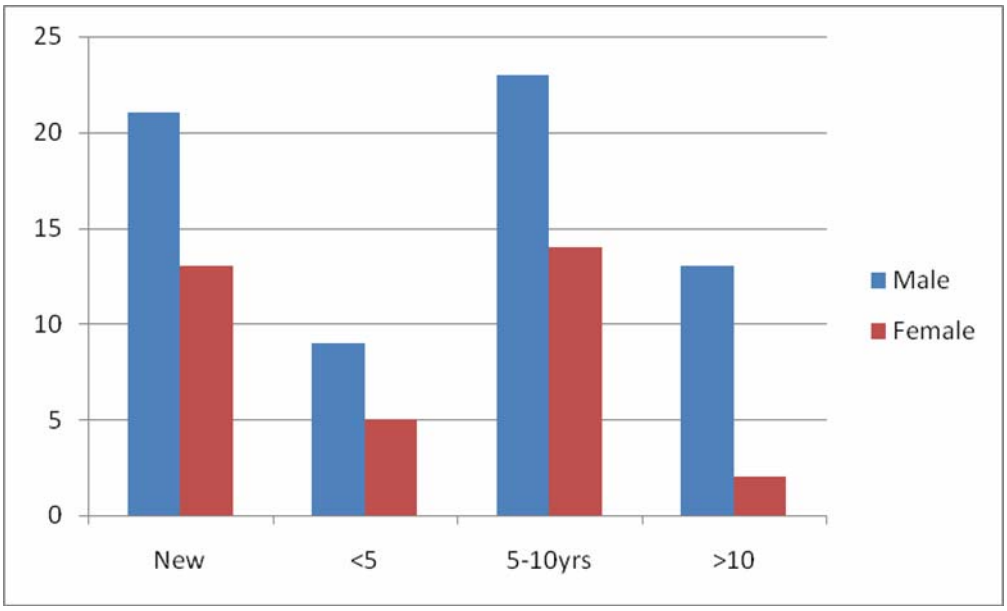


In our study, polydipsia was the most common symptom, followed by polyuria,polyphagia,visual disturbances &oliguria.

Table :5 Duration of diabetes

Duration	Total		Male		Female	
	No.	%	No.	%	No.	%
New	34	34	21	61	13	39
<5	14	14	9	64	5	36
5-10yrs	37	37	23	62	14	38
>10	15	15	13	86	2	14

Chart showing the duration of diabetes

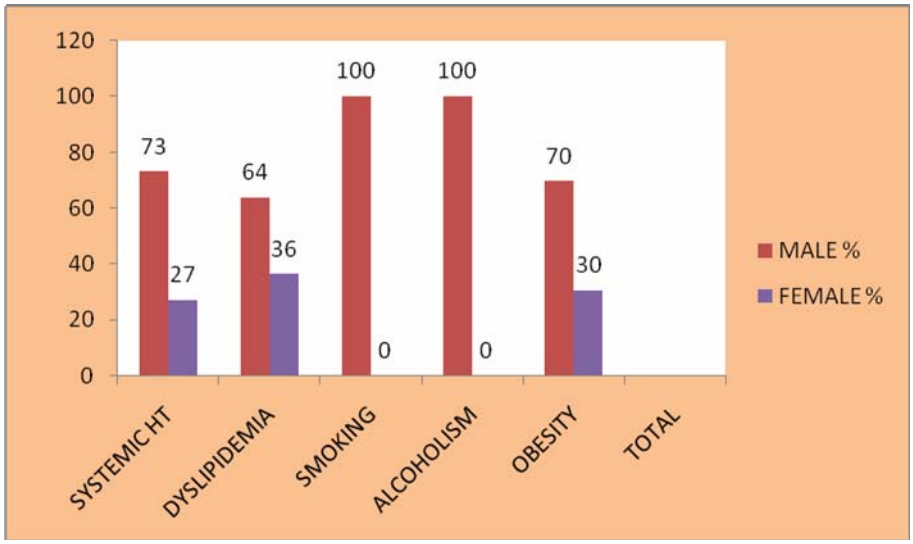


In our study, 34% patients were newly diagnosed DM,of which 61% were males,39% were females.14% patients were diabetics of < 5years duration.37% had a duration of 5 to 10 years and 15% had diabetes of more than 10 years.

Table: 6 Risk factors

Risk factors	***Total***	***Male***		***Female***	
	No.	***No.***	***%***	***No.***	***%***
Systemic hypertension	26	19	73	7	27
Dyslipidemia	11	7	64	4	36
Smoking	22	22	100	0	0
Alcoholism	13	13	100	0	0
Obesity	23	16	70	7	30
Total	**95**	**77**		**18**	

Chart showing the various risk factors among patients

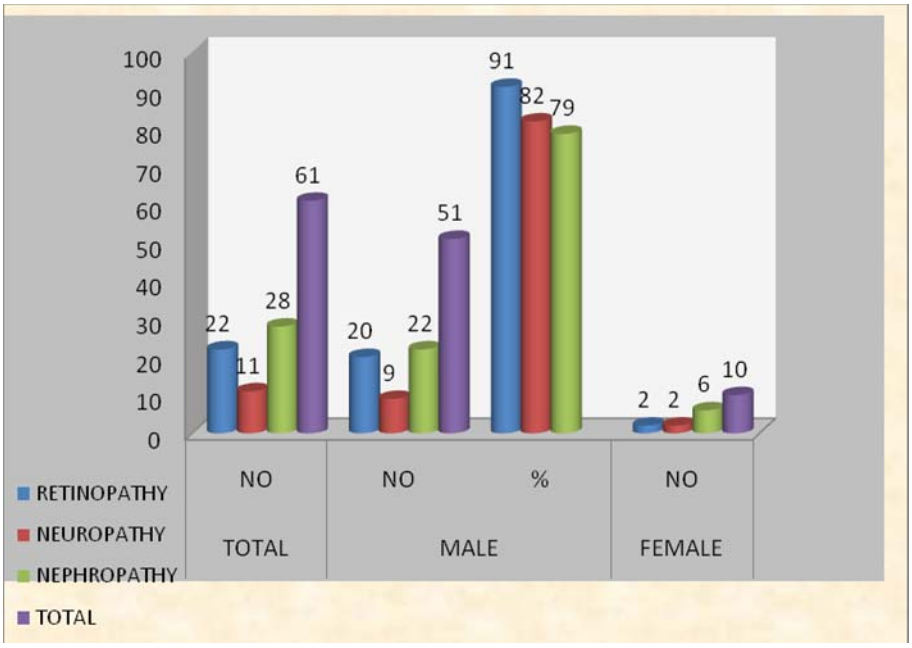


In our study, most common risk factor was systemic hypertension 26%, followed by obesity[23%], smoking[22%], alcoholism[13%] & dyslipidemia[11%].

Table: 7 Microvascular complications

	Total	Male		Female	
	No.	**No.**	**%**	**No.**	**%**
Retinopathy	22	20	91	2	9
Neuropathy	11	9	82	2	18
Nephropathy	28	22	79	6	21
Total	**61**	**51**		**10**	

Chart showing the various microvascular complications

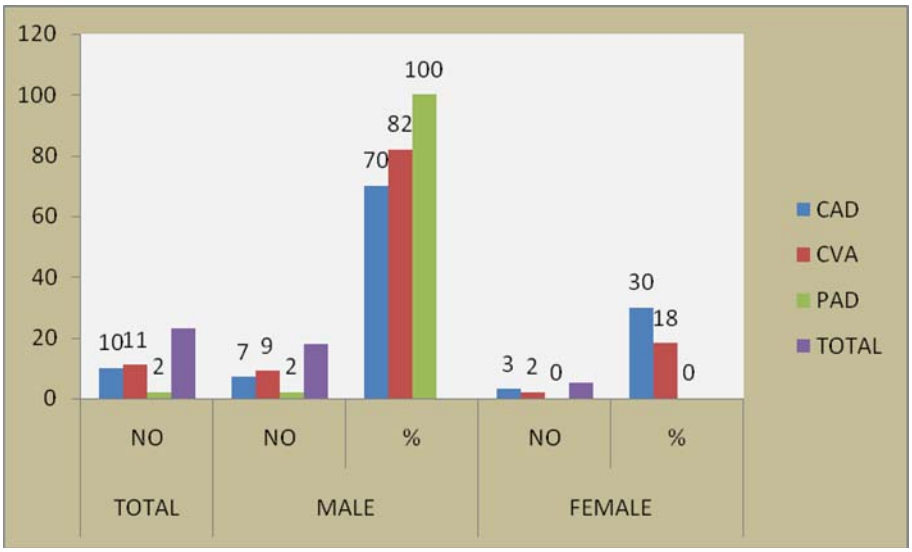


The most common micro vascular complication was nephropathy [28%] of patients,followed by Retinopathy [22%] of patients.

Table: 8 Macrovascular complications

	Total	Male		Female	
	No.	No.	%	No.	%
CAD	10	7	70	3	30
CVA	11	9	82	2	18
PAD	2	2	100	0	0
Total	**23**	**18**		**5**	

C hart showing the various macrovascular complications

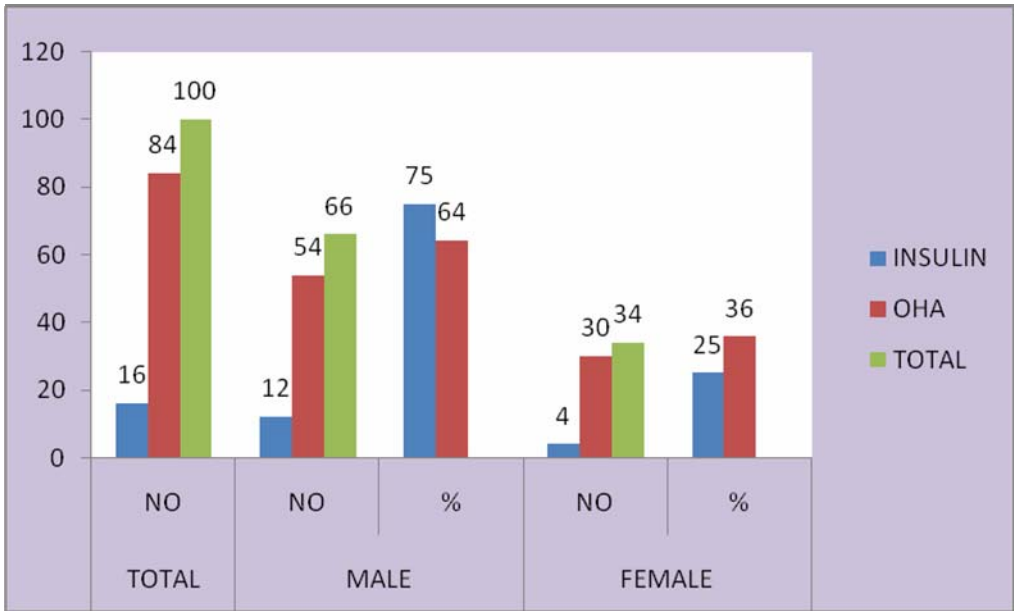


In our study, the most common macro vascular complication was stroke, which was present in 11% patients,followed by coronary artery disease,seen in 10% patients.

Table: 9 Treatment regimen

	Total	Male		Female	
	No.	**No.**	**%**	**No.**	**%**
Insulin	16	12	75	4	25
OHA	84	54	64	30	36
Total	100	66		34	

Chart showing the treatment regimen



In our study, 84% patients was on treatment with OHA, out of which 64% were male,36% were female.16% patients were getting insulin treatment.

Table: 10 Comparison of serum zinc levels between patients and control

Parameter	Subject (n=100)	Control (n=50)	P value
Mean Age	47	48	0.6379
Mean Zinc Levels	63.08	75.48	0.0001

In our study, 100 patients when compared to 50 controls had significant lower levels of zinc with a p value of <0.0001.

Table:11 Serum zinc levels in males and females

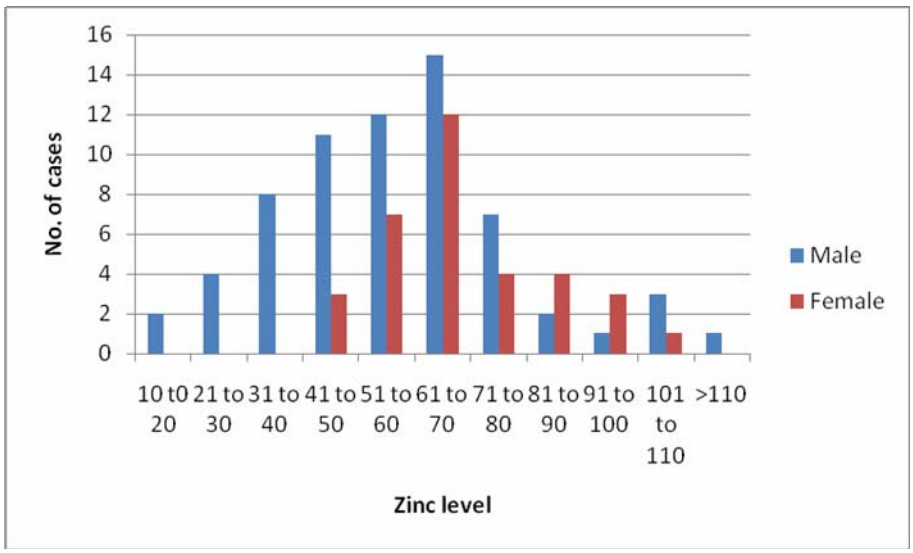
Zinc Levels[µg]	*Total*	*Male*		*Female*	
		No.	*%*	*No.*	*%*
10 - 20	2	2	100	0	0
21- 30	4	4	100	0	0
31 - 40	8	8	100	0	0
41- 50	14	11	78	3	22
51 - 60	19	12	63	7	37
61 - 70	27	15	55	12	45
71 - 80	11	7	63	4	37
81 - 90	6	2	33	4	67
91- 100	4	1	25	3	75
101 - 110	4	3	75	1	25
>110	1	1	100	0	0
TOTAL	**100**	**66**		**34**	

In our study, 100 patients when compared to 50 controls had significant lower levels of zinc with a p value of <0.0001.

Table:11 Serum zinc levels in males and females

Zinc Levels[µg]	Total	Male		Female	
		No.	%	No.	%
10 - 20	2	2	100	0	0
21- 30	4	4	100	0	0
31 - 40	8	8	100	0	0
41- 50	14	11	78	3	22
51 - 60	19	12	63	7	

Chart showing the serum zinc levels in males and females

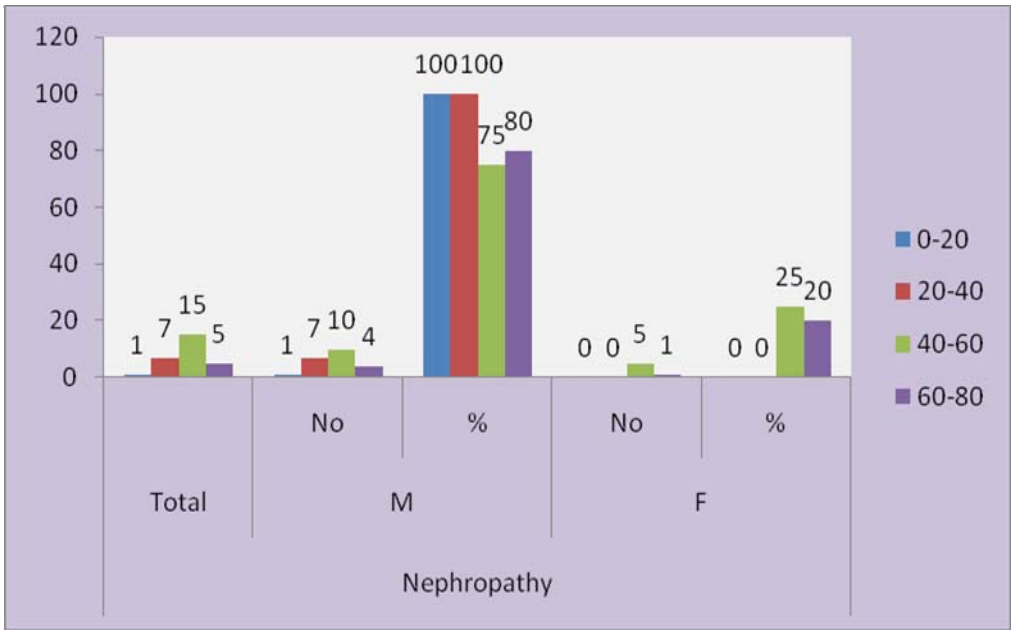


In our study, it was found that 47 people had low levels of Zinc (<60 micrograms) of which majority(19) belonged to the 51-60 micrograms category. 2 persons had values in the range of 10-20 µg .

Table:12 Zinc level in nephropathy

Zinc level (µg)	*Nephropathy*				
	Total	*Males*		*Females*	
		No.	*%*	*No.*	*%*
0-20	1	1	100	0	0
20-40	7	7	100	0	0
40-60	15	10	75	5	25
60-80	5	4	80	1	20

Chart showing zinc level in nephropathy

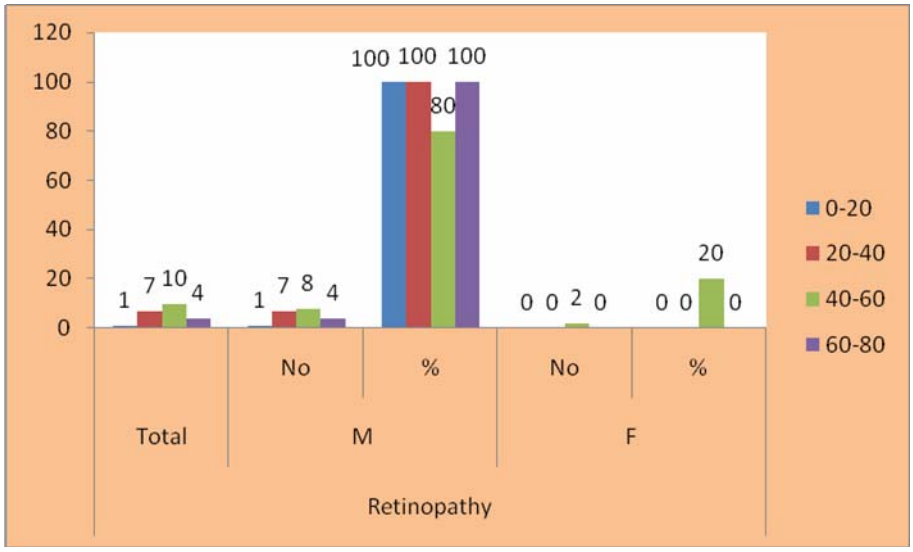


In our study, patients with nephropathy formed 28%,of which 82% had low serum zinc level[<60 micro gram]

Table:13 Zinc level in Retinopathy

Zinc level (µg)	***Retinopathy***				
	Total	***Males***		***Females***	
		No.	***%***	***No.***	***%***
0-20	1	1	100	0	0
20-40	7	7	100	0	0
40-60	10	8	80	2	20
60-80	4	4	100	0	0

Chart showing zinc level in retinopathy

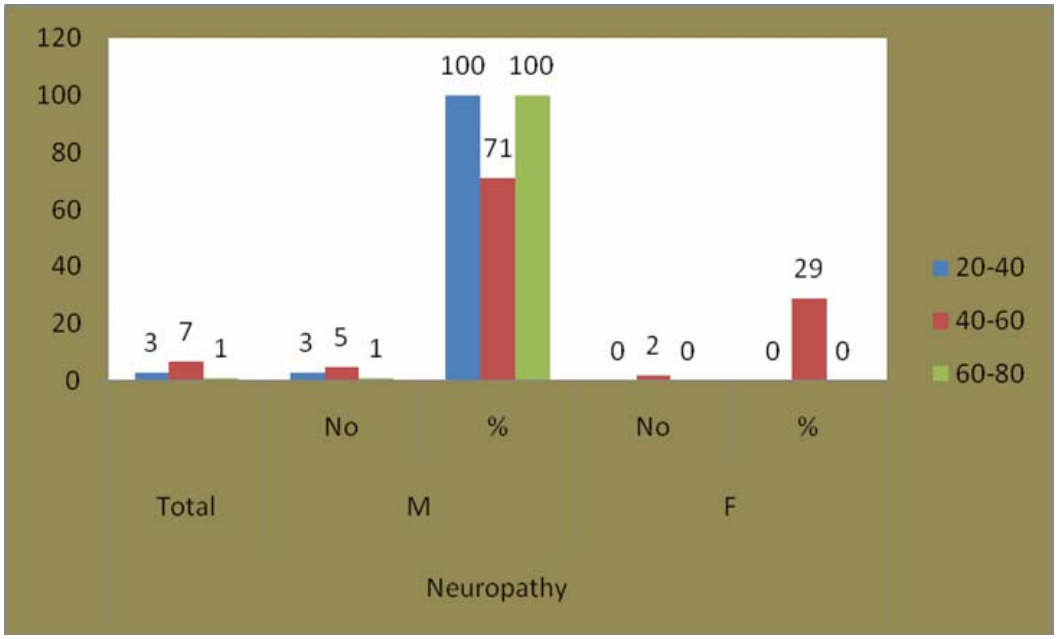


In our study, patients with Retinopathy formed 22%,of which 81% had low serum zinc levels[<60 micro gram].

Table: 14 Zinc level in neuropathy

Zinc level (µg)	*Neuropathy*				
	Total	*Males*		*Females*	
		No.	*%*	*No.*	*%*
20-40	3	3	100	0	0
40-60	7	5	71	2	29
60-80	1	1	100	0	0

Chart showing zinc level in neuropathy

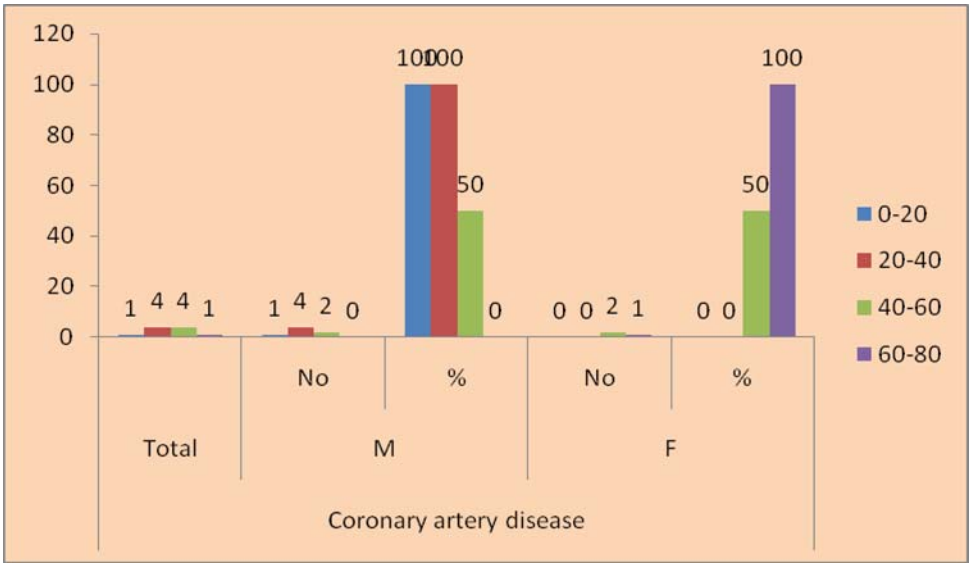


In our study, patients with neuropathy formed 11%,of which 90% had low serum zinc levels.[<60 micro gram]

Table: 15 Zinc level in CAD

Zinc level (µg)	*Coronary artery disease*				
	Total	*Males*		*Females*	
		No.	*%*	*No.*	*%*
0-20	1	1	100	0	0
20-40	4	4	100	0	0
40-60	4	2	50	2	50
60-80	1	0	0	1	100

Chart showing zinc level in CAD

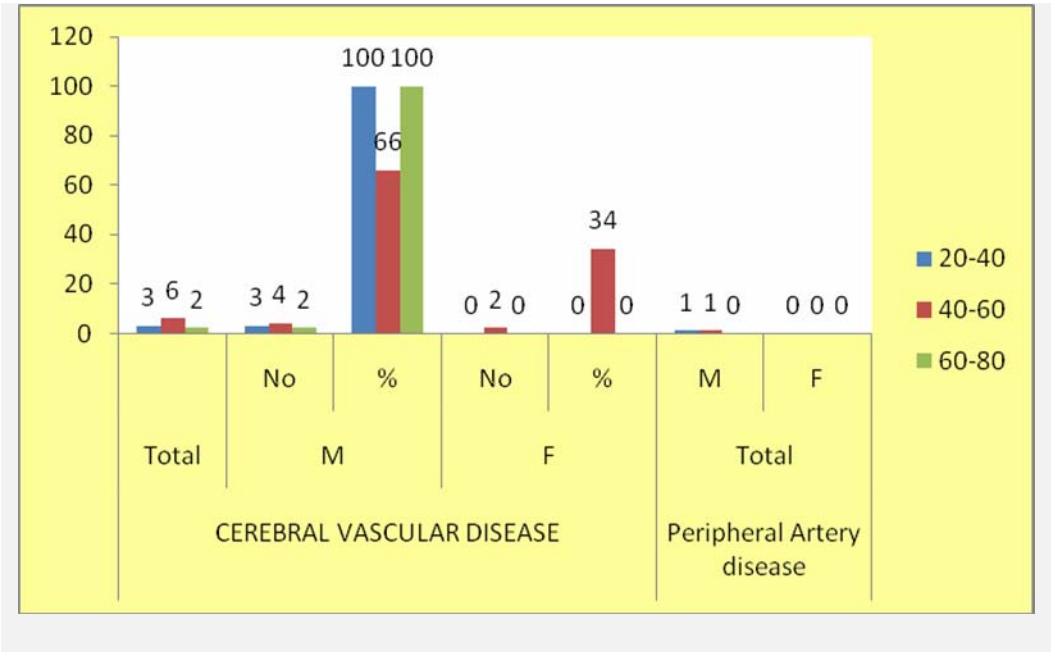


In our study, patients with CAD formed10%, of which 90% had low serum zinc levels.

Table: 16 Zinc level in CVA and PAD

Zinc level (µg)	*Cerebro vascular disease*					*Peripheral Artery disease*	
	Total	*Males*		*Females*		*Total*	
		No.	*%*	*No.*	*%*	*M*	*F*
20-40	3	3	100	0	0	1	0
40-60	6	4	66	2	34	1	0
60-80	2	2	100	0	0	0	0

Chart showing zinc level in CVA and PAD

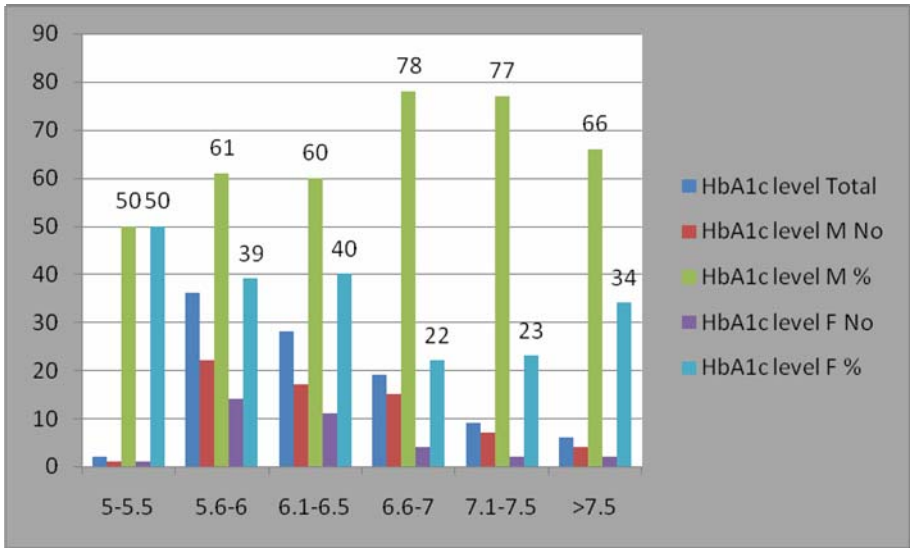


In our study, patients with CVA formed 11%,of which 81% had low serum zinc levels.Patients with PAD formed 2%, all had low serum zinc levels.

Table: 17 HbA1c level in males and females

HbA1c level %	***Total***	***Males***		***Females***	
		No.	***%***	***No.***	***%***
5-5.5	2	1	50	1	50
5.6-6	36	22	61	14	39
6.1-6.5	28	17	60	11	40
6.6-7	19	15	78	4	22
7.1-7.5	9	7	77	2	23
>7.5	6	4	66	2	34

Chart showing HbA1C level in males and females



In our study, 36% had HbA1c level in the range of 5.6 - 6,followed by 28% who had HbA1C Level in the range of 6.1-6.5.

Table: 18 Correlation of zinc level in micro &macro vascular complications

Parameter	*Microvascular*	*Macrovascular*
No.of patients	61	23
Mean Zn level (µg)	46.52	43.86

In our study patients with micro vascular complications, there was no significant variaton in serum zinc levels in each groups [retinopathy,nephropathy&neuropathy] with p value of 0.8431.In Patients with macro vascular complications also, there was no significant variation in serum zinc level in each groups[CAD,CVA&PAD] with p value of 0.4537.

Table: 19 Comparision of zinc level and HbA1c level

HbA1c level	*Total No.of patients*	*Mean Zinc level*
5-5.5	2	78
5.6-6	36	70
6.1-6.5	28	64
6.6-7	19	52
7.1-7.5	9	50
>7.5	6	46

In our study, there was a significant relation between serum HbA1C level and zinc levels.Patients with poor glycaemic control had low serum zinc level compared to patients with good glycaemic control,which was statistically significant with p value of <0.0001.

Table 20: Comparision of serum zinc level with duration of DM

Duration	***No.of patients***	***Serum mean zinc level***	
Newly diagnosed	34	69.74	p value<0.05
1-4 years	14	69.78	
5-10 years	37	56.16	
>10 years	15	51.93	

Patients with long standing diabetes had lower zinc levels than newly diagnosed patients, which was found to be statistically significant with p value < 0.0035.

DISCUSSION

The total number of patients analysed were 100,out of which 66% were males and 34% were females.The prevalence of diabetes was found to increase as the age advances.

This finding correlated with the study done by Ahuja mms[1996].Most of the patients in the study group were in the age group of 51-60 years[35%].85% of patients had type 2DM,of which 65% were males and 35% were females.

26% of patients in our study group had systemic hypertension, obesity was found in 23% of patients. Other risk factors like smoking [22%], alcohol [13%]& dyslipidemia [11%] were also present.

Of the 100 patients in the study, 84 were on Oral Hypoglycemic Agents and the remaining 16 were on Insulin therapy.

23 % of the subjects had macrovascular complications in the form of CAD (10%), CVA (11%) and PAD(2%)

61% of the subjects had Micro vascular complications in the form of Nephropathy (28%), retinopathy (22%) and Neuropathy (11%)

The Mean Value for Serum Zinc levels in healthy individuals studied was within normal range as established by Study of Zinc Homeostasis in Humans [72]. Likewise our study showed decreased levels of Serum Zinc in Diabetic individuals compared to normal subjects. The minimum Zinc value found in the present study was similarly reported by a number of other studies [73], [74], [75]

It was also found in our study that compared to the newly diagnosed, patients with long standing Diabetes Mellitus had lower levels of Zinc.

Among the subjects with microvascular complications, 90% with neuropathy, 82% of those with nephropathy, 81% with retinopathy had low serum Zinc levels.

But there was no significant statistical variation in serum zinc levels between the various micro vascular complications.

Among the subjects with macrovascular complications 100% with PAD, 90% with CAD and 81% with CVA had lower levels of Zinc.

But there was nosignificant statistical variation in serum zinc levels between the various macro vascular complications.

Similarly there was no significant statistical variation in serum zinc levels between the macro vascular and microvascular complications.

There was a significant negative correlation between HbA1C and serum Zinc levels.

Also, patients with poor glycemic control had lower Zinc levels compared to the subjects with a better glycemic control. This correlated with the conclusions of a study done by Al-Maroof and Al-Sabatti in 2006 for the Ministry of Health, Iraq.

LIMITATIONS OF THE STUDY

- Zinc Supplementation was not done hence its effect on the subjects could not be ascertained.
- Urinary and Stool Zinc levels were not measured.

CONCLUSION

- Diabetes is more commonly seen in males (66%) compared to females (34%).
- Diabetes is commonly diagnosed in the age group of 51-60 yrs.
- Among those with Diabetes, the microvascular complications (61%) occur more often than the macrovascular complications (23%).
- Nephropathy (28%) is the commonest microvascular complication closely followed by Retinopathy (22%).
- Diabetic individuals have significantly lower levels of Zinc when compared to normal healthy individuals.
- Patients with long standing DM have lower Zinc levels than those who are newly diagnosed.
- Patients with poor glycemic control have lower Zinc levels compared to the subjects with a better glycemic control.

Zinc supplementation may have a therapeutic role in control and prevention of complications in DM. Further studies are needed to clarify this aspect.

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PROFORMA

NAME

AGE

SEX

I.P NO

OCCUPATION & ADDRESS

DURATION OF DIABETES MELLITUS:

SYMPTOMS

Polyuria	Oliguria	Visual disturbances
Polydypsia	Puffiness of Face	Weight loss/gain
Polyphagia	Swelling of legs	High coloured urine
Giddiness	Anorexia	Headache/ LOC/
Seizures	Altered sensorium	
Chest Pain	Vomiting/Hiccup	Sensory disturbances
Palpitation	Easy fatiguability	Poor wound healing
Leg ulcers		
Dyspnea	Altered bowel habit	Limb weakness
Fever	Dysuria	Abdominal Pain

PAST HISTORY

- * Hypertension * Renal disorders *CAD
- * CVA *Seizure disorder * Pulmonary TB
- *Surgical illness * Blood transfusion *Hypoglycemic attacks
- *Thyroid Disorder * Liver disorders
- * Drug intake- Ethambutol ,Penicillamine, Iron, Dietary fibres, Sodium valproate

PERSONAL HISTORY

- Smoking
- Alcoholism
- Drug abuse
- STD
- Prolonged Starvation
- Exercise

ANTHROPOMETRY

- Ht: cms Wt: kgs BMI:
- Hydration Status JVP

FUNDUS

- Features of Thyroid Disorders:
- Features of Cushing's Syndrome:

- **Pulse:**
- **Blood Pressure:**

  (Stage of Hypertension)

SYSTEMIC EXAMINATION:

CVS:

RS:

Abdomen:

CNS:

INVESTIGATIONS

- Hb: TC: DC: P- L- E- M- Plt:
- ESR :
- Blood Glucose Fasting : Blood Glucose PP:
- Blood Urea :
- Serum Creatinine :
- Uric Acid :
- Serum Electrolytes: Na+ K + HCO3-
- Lipid Profile:
- Total Cholesterol : HDL :
- Triglycerides : LDL :
- Chol: HDL Ratio : VLDL :
- Liver function test:

- Bilirubin Total :

  Direct :
- SGOT :
- SGPT :
- Sr. Alkaline Phosphatase :
- Total Protein :

  Albumin :

  Globulin :

Urine examination:

- Micro Albumin : Deposits:
- Albumin :
- Sugar :
- ECG:

X-ray chest pa view :

- Echo cardiogram :
- USG abdomen :
- HIV serology :
- CT brain (if indicated) :
- Carotid & vertebral doppler (if indicated) :
- Nerve conduction study (if indicated) :
- Serum zinc level:

MASTER CHART

NO	NAME	AGE	SEX	NEW/OLD	Symptoms										TYPE	DURATION(YEARS)	TREATMENT(OHA/INSULIN)	SMOKING	ALCOHOLISM	SYSTEMIC HT	Complications								FBS	PPBS	HBA1C	SERUM ZINC LEVEL(µg/dl)
					POLYURIA	POLYPHAGIA	POLYDYPSIA	BREATHLESSNESS	CHESTPAIN	OLIGURIA	VISUAL DISTURBANCES	SENSORY MOTOR DISTURBANCES	OBESITY	LEG ULCERS							RETINOPATHY	CVA	MICROALBUMINURIA	CAD	PERIPHERAL ARTERIAL DISEASE	NEUROPATHY	DYSLIPIDEMIA	ABNORMAL RENAL FUNCTION				
1	GOVINDARAJ	57	M	N	P	P	P	A	A	A	A	A	A	A	2	NIL	OHA	A	A	P	A	A	A	A	A	A	A	A	112	152	6	56
2	SUBBU	51	M	N	P	P	P	A	A	A	A	A	A	A	2	NIL	OHA	A	A	P	A	A	A	A	A	A	A	A	142	181	6.5	62
3	KOILRAJ	32	M	N	P	P	P	A	A	A	A	A	A	A	2	NIL	OHA	A	A	A	A	A	A	A	A	A	A	A	121	177	6.3	77
4	VENKATRAM	46	M	O	A	A	A	A	A	A	A	A	A	A	2	10	INS	A	A	A	A	A	A	A	A	A	A	A	156	233	7.5	52
5	ARUMUGAM	48	M	O	A	A	A	A	A	A	A	A	A	A	2	5	OHA	A	A	A	A	A	A	A	A	A	A	A	132	246	7.3	64
6	ARUNACHALAM	23	M	N	P	P	P	A	A	A	A	A	A	A	1	NIL	INS	A	A	A	A	A	A	A	A	A	A	A	160	252	8.2	45
7	RAJA	42	M	O	A	A	P	A	A	A	A	A	A	A	2	3	OHA	A	A	A	A	A	A	A	A	A	A	A	144	198	7.6	48
8	KANNAN	56	M	N	P	A	P	A	A	A	A	A	A	A	2	NIL	OHA	A	A	A	A	A	A	A	A	A	A	A	129	160	6.9	50
9	RASU	69	M	O	A	A	A	A	A	A	A	P	A	A	2	16	OHA	A	A	P	A	A	A	A	A	P	A	A	119	198	6.8	55
10	SUBRAMANI	51	M	N	P	P	P	A	A	A	A	A	A	A	2	NIL	OHA	A	A	A	A	A	A	A	A	A	A	A	154	211	7.2	79
11	MAHENDRAN	32	M	N	P	P	P	A	A	A	A	A	A	A	2	NIL	OHA	P	A	A	A	A	A	A	A	A	A	A	166	208	7	80
12	LAKSHMI	42	F	N	P	P	P	A	A	A	A	A	A	A	2	NIL	OHA	A	A	P	A	A	A	A	A	A	A	A	134	186	6.7	68
13	BAGYAVADHI	26	F	O	A	A	A	A	A	A	A	A	A	A	1	10	INS	A	A	A	A	A	A	A	A	A	A	A	155	206	6.1	61
14	MURUGAN	72	M	O	A	A	P	A	A	A	A	A	A	A	2	25	OHA	P	A	A	A	A	A	A	A	A	A	A	134	178	6.8	59
15	KRISHNAN	39	M	N	P	P	P	A	A	A	A	A	A	A	2	NIL	OHA	A	A	A	A	A	A	A	A	A	A	A	143	173	5.9	59
16	KADHIRESAN	61	M	O	A	A	A	A	A	P	A	A	P	A	2	14	OHA	A	A	A	A	A	A	A	A	A	A	P	119	165	6.7	61
17	MARY	48	F	N	P	P	P	A	A	A	A	A	P	A	2	NIL	OHA	A	A	P	A	A	A	A	A	A	A	A	124	149	5.9	69
18	RAVI	14	M	N	P	P	A	A	A	A	A	A	A	A	1	NIL	INS	A	A	A	A	A	A	A	A	A	A	A	133	167	7.2	29
19	VIJAYASANKARAN	49	M	O	A	A	A	A	A	A	A	A	A	A	2	8	OHA	A	A	A	A	A	A	A	A	A	A	A	124	154	6.1	79
20	GOWRIAMMAL	59	F	N	A	A	P	A	A	A	A	A	A	A	2	NIL	OHA	A	A	P	A	A	A	A	A	A	A	A	169	244	8.9	51
21	DHANAPAL	34	M	O	A	A	A	P	P	A	A	A	A	A	1	20	INS	P	A	A	A	A	A	P	A	A	A	A	145	176	6.7	34
22	MARKANDEYAN	51	M	N	P	A	P	A	A	A	A	A	A	A	2	NIL	OHA	A	A	A	A	A	A	A	A	A	A	A	140	189	6.2	77
23	KUPPUSAMY	68	M	O	A	A	A	A	A	A	A	A	A	A	2	15	OHA	A	A	P	A	A	A	A	A	A	A	A	117	134	5.8	67
24	THILAGAVATHY	49	F	N	P	P	A	A	A	A	A	A	A	A	2	NIL	OHA	A	A	A	A	A	A	A	A	A	A	A	129	177	6.9	70

NO	NAME	AGE	SEX	NEW/OLD	Symptoms										TYPE	DURATION(YEARS)	TREATMENT(OHA/INSULIN)	SMOKING	ALCOHOLISM	SYSTEMIC HT	Complications								FBS	PPBS	HBA1C	SERUM ZINC LEVEL(µg/dl)
					POLYURIA	POLYPHAGIA	POLYDYPSIA	BREATHLESSNESS	CHESTPAIN	OLIGURIA	VISUAL DISTURBANCES	SENSORY MOTOR DISTURBANCES	OBESITY	LEG ULCERS							RETINOPATHY	CVA	MICROALBUMINURIA	CAD	PERIPHERAL ARTERIAL DISEASE	NEUROPATHY	DYSLIPIDEMIA	ABNORMAL RENAL FUNCTION				
25	RAMASAMY	53	M	O	A	A	A	P	P	A	A	A	A	A	2	13	OHA	P	A	P	A	A	A	P	A	A	P	P	144	189	6.3	38
26	SENTHILNATHAN	22	M	O	A	A	A	A	A	A	A	A	A	A	1	5	INS	A	A	A	A	A	A	A	A	A	A	A	132	176	6	69
27	RAJA SEKAR	43	M	O	A	A	A	P	A	P	A	A	P	A	2	7	OHA	A	P	A	A	P	A	P	A	A	P	P	126	188	6.9	44
28	ROSELIN MARY	37	F	N	P	P	A	A	A	A	A	A	P	A	2	NIL	OHA	A	A	P	A	A	A	A	A	A	A	A	129	155	5.9	89
29	ANTONY	41	M	N	A	A	P	A	A	A	A	A	A	A	2	NIL	OHA	A	A	A	A	A	A	A	A	A	A	A	119	177	6.4	51
30	FATHIMA BEE	59	F	N	P	P	P	A	A	A	A	A	A	A	2	NIL	OHA	A	A	P	A	A	A	A	A	A	A	A	168	210	8	50
31	PARAMESWARAN	18	M	N	P	P	P	A	A	A	A	A	A	A	1	NIL	INS	A	A	A	A	A	A	A	A	A	A	A	188	266	9	46
32	RAJAIYAH	59	M	N	A	A	A	A	A	A	A	A	P	A	2	NIL	OHA	A	P	A	A	A	A	A	A	A	A	A	135	176	7	107
33	VARALAKSHMI	38	F	N	P	P	P	A	A	A	A	A	P	A	2	NIL	OHA	A	A	P	A	A	A	A	A	A	A	A	121	155	6.3	78
34	PERUMAL	49	M	N	P	P	A	A	A	A	A	A	A	A	2	NIL	OHA	A	A	P	A	A	A	A	A	A	A	A	119	145	6	80
35	GANDHIMATHI	55	F	N	A	A	A	A	A	A	A	A	A	A	2	NIL	OHA	A	A	A	A	A	A	A	A	A	A	A	119	178	7.1	51
36	KUMARAN	25	M	O	A	A	A	A	A	A	A	A	A	A	1	9	INS	A	A	A	A	A	A	A	A	A	A	A	110	143	5.8	62
37	CHINNAMA	67	F	O	A	A	A	P	P	A	A	A	A	A	2	7	OHA	A	A	A	A	A	A	P	A	A	P	A	142	177	6.9	42
38	AYYAPAN	29	M	N	P	P	P	A	A	A	A	A	A	A	1	NIL	INS	A	A	A	A	A	A	A	A	A	A	A	131	158	6.2	70
39	MALLIGA	46	F	N	P	P	P	A	A	A	A	A	P	A	2	NIL	OHA	A	A	A	A	A	A	A	A	A	A	A	139	171	6	109
40	RAMALINGAM	56	M	N	P	A	A	A	A	A	A	A	A	A	2	NIL	OHA	A	A	P	A	A	A	A	A	A	A	A	122	155	8.1	38
41	KAMARAJ	42	M	N	P	P	P	A	A	A	A	A	P	A	2	NIL	OHA	A	A	A	A	A	A	A	A	A	A	A	119	149	6	70
42	DHANAM	39	F	O	A	A	A	A	A	A	A	A	A	A	2	1	OHA	A	A	A	A	A	A	A	A	A	A	A	110	158	6.1	95
43	VENKATACHALAM	51	M	O	A	A	A	P	A	P	A	A	P	A	2	16	OHA	P	P	P	A	A	A	P	A	A	P	P	155	190	7.3	18
44	GANESAN	52	M	O	A	A	A	A	A	A	A	A	A	A	2	11	OHA	A	A	P	A	A	A	A	A	A	A	A	118	175	6.2	94
45	MOORTHY	54	M	O	A	A	A	A	A	A	P	A	A	A	2	7	OHA	A	P	P	P	A	P	A	A	A	A	A	102	152	6.8	25
46	SANKARI	19	F	O	A	A	A	A	A	A	A	A	A	A	1	13	INS	A	A	A	A	A	P	A	A	A	A	A	114	137	5.3	55
47	SASIKALA	29	F	O	A	A	A	A	A	A	A	A	A	A	1	11	INS	A	A	A	A	A	A	A	A	A	A	A	102	130	5.8	79
48	SIVAKUMAR	38	M	N	P	P	P	A	A	A	A	A	P	A	2	NIL	OHA	A	A	P	A	A	A	A	A	A	A	A	116	139	6	112
49	MANIKANDAN	41	M	N	A	P	P	A	A	A	A	A	A	A	2	NIL	OHA	A	P	P	A	A	A	A	A	A	A	A	127	164	6.3	62

NO	NAME	AGE	SEX	NEW/OLD	Symptoms															Complications												
					POLYURIA	POLYPHAGIA	POLYDYPSIA	BREATHLESSNESS	CHESTPAIN	OLIGURIA	VISUAL DISTURBANCES	SENSORY MOTOR DISTURBANCES	OBESITY	LEG ULCERS	TYPE	DURATION(YEARS)	TREATMENT(OHA/INSULIN)	SMOKING	ALCOHOLISM	SYSTEMIC HT	RETINOPATHY	CVA	MICROALBUMINURIA	CAD	PERIPHERAL ARTERIAL DISEASE	NEUROPATHY	DYSLIPIDEMIA	ABNORMAL RENAL FUNCTION	FBS	PPBS	HBA1C	SERUM ZINC LEVEL(µg/dl)
50	AANDAWAR	78	M	O	A	A	A	A	A	A	A	A	A	A	2	5	OHA	A	A	A	A	A	A	A	A	A	A	A	A	119	169	6	54
51	MURUGESWARI	53	F	N	P	P	P	A	A	A	A	A	A	A	2	NIL	OHA	A	A	A	A	A	A	A	A	A	A	A	A	104	149	6.9	53
52	BALAMURUGAN	23	M	O	A	A	A	A	A	A	P	A	A	A	1	13	INS	P	A	A	P	A	P	A	A	A	A	A	A	110	166	6.4	39
53	JAYALAKSHMI	51	F	N	P	P	P	A	A	A	A	A	A	A	2	NIL	OHA	A	A	A	A	A	A	A	A	A	A	A	A	102	155	5.9	90
54	VIGNESHWARAN	17	M	O	A	A	P	A	A	A	P	A	A	A	1	12	INS	A	A	A	P	A	P	A	A	A	A	A	A	117	169	6.7	41
55	SARASWATHY	65	F	O	A	A	A	A	A	A	A	A	A	A	2	6	OHA	A	A	A	A	A	A	A	A	A	A	A	A	123	147	5.9	69
56	ROSAMMAL	57	F	O	A	A	A	A	A	A	A	A	A	A	2	2	OHA	A	A	A	A	A	A	A	A	A	A	A	A	111	136	6.1	68
57	THIYAGARAJAN	59	M	O	A	A	A	A	A	A	A	A	A	A	2	3	OHA	A	A	A	A	A	A	A	A	A	A	A	A	109	146	5.8	62
58	AYYASAMY	57	M	O	A	A	A	A	A	A	P	A	P	A	2	11	OHA	A	A	P	P	A	P	A	A	A	A	A	A	125	189	6.8	26
59	PAPPAMMAL	69	F	O	A	A	A	A	A	A	A	A	A	A	2	6	OHA	A	A	A	A	A	A	A	A	A	A	A	A	112	155	6.3	64
60	BALAJI	37	M	O	A	A	A	A	A	A	P	A	A	A	2	5	OHA	P	P	P	P	P	P	A	A	A	A	A	A	120	189	7.1	51
61	VIDHYA	41	F	O	A	A	A	A	A	A	A	A	A	A	2	1	OHA	A	A	A	A	A	A	A	A	A	A	A	A	131	167	5.9	68
62	SARAVANAN	43	M	O	A	A	A	P	A	P	A	P	A	P	2	8	OHA	P	A	A	A	P	P	P	A	P	P	A	A	121	149	6.2	31
63	SUDHAKAR	39	M	O	A	A	A	A	A	A	P	A	A	A	2	6	OHA	P	A	A	P	P	P	A	A	A	A	A	A	109	143	5.9	67
64	SANGEETHA	44	F	O	A	A	P	A	A	A	A	P	A	P	2	5	OHA	A	A	A	A	A	P	A	A	P	A	A	A	100	139	6.1	56
65	USHA	51	F	N	P	P	P	A	A	A	A	A	A	A	2	NIL	OHA	A	A	A	A	A	A	A	A	A	A	A	A	98	139	5.8	99
66	GOPAL	44	M	O	A	A	A	P	P	P	A	A	P	A	2	2	OHA	A	A	A	A	P	A	P	A	A	P	A	A	122	146	6.1	46
67	SUBRAMANI	51	M	O	A	A	A	A	A	A	P	P	P	P	2	9	OHA	P	A	A	P	A	P	A	P	P	A	A	A	113	142	6	51
68	STALIN	29	M	O	A	A	A	A	A	A	P	A	A	A	1	16	INS	A	A	A	P	A	P	A	A	A	A	A	A	105	138	5.8	69
69	KANNADHASAN	56	M	O	A	A	A	A	A	A	P	P	A	P	2	7	OHA	P	A	A	P	A	P	A	P	P	A	A	A	133	178	6.9	31
70	KASTHOORI	28	F	O	A	A	A	A	A	A	A	A	A	A	1	5	INS	A	A	A	A	A	A	A	A	A	A	A	A	113	166	5.9	99
71	ESWARAN	64	M	N	P	A	P	A	A	A	A	A	A	A	2	NIL	OHA	A	A	P	A	A	A	A	A	A	A	A	A	100	132	5.6	78
72	GUNASEKARAN	59	M	O	A	A	A	A	A	A	P	P	A	A	2	10	OHA	P	P	A	P	P	P	A	A	P	A	A	A	101	169	7	46
73	JOTHI	37	F	O	A	A	A	A	A	A	A	A	A	A	2	2	OHA	A	A	A	A	A	A	A	A	A	A	A	A	112	145	6.4	68
74	VARADHARAJAN	49	M	O	A	A	P	A	A	A	A	A	P	A	2	4	OHA	A	A	A	A	A	P	A	A	A	A	A	A	122	150	6.1	59

NO	NAME	AGE	SEX	NEW/OLD	Symptoms															Complications												
					POLYURIA	POLYPHAGIA	POLYDYPSIA	BREATHLESSNESS	CHESTPAIN	OLIGURIA	VISUAL DISTURBANCES	SENSORY MOTOR DISTURBANCES	OBESITY	LEG ULCERS	TYPE	DURATION(YEARS)	TREATMENT(OHA/INSULIN)	SMOKING	ALCOHOLISM	SYSTEMIC HT	RETINOPATHY	CVA	MICROALBUMINURIA	CAD	PERIPHERAL ARTERIAL DISEASE	NEUROPATHY	DYSLIPIDEMIA	ABNORMAL RENAL FUNCTION	FBS	PPBS	HBA1C	SERUM ZINC LEVEL(µg/dl)
75	SEENIAMMAL	79	F	O	A	A	A	A	A	A	A	A	P	A	2	7	OHA	A	A	A	A	A	A	A	A	A	A	A	110	146	6.2	79
76	LATHA	47	F	O	A	A	A	P	P	P	A	P	P	A	2	6	OHA	A	A	A	A	P	P	P	A	P	P	A	115	139	5.9	56
77	DAMODHARAN	61	M	N	P	P	P	A	A	A	A	A	A	A	2	NIL	OHA	P	A	P	A	A	A	A	A	A	A	A	112	177	5.6	88
78	KARUNANIDHI	49	M	O	A	A	A	A	A	A	P	A	A	A	2	9	OHA	P	P	A	P	P	P	A	A	A	A	A	105	138	6	25
79	THANGIRAMU	52	F	N	A	P	P	A	A	A	A	A	P	A	2	NIL	OHA	A	A	A	A	A	A	A	A	A	A	A	89	125	6	80
80	ALAGESAN	54	M	O	A	A	A	A	A	A	A	A	A	A	2	5	OHA	P	A	P	P	A	P	A	A	A	A	A	95	134	5.8	47
81	PAULRAJ	36	M	O	A	A	A	A	A	A	P	P	A	P	2	4	OHA	P	A	A	P	A	P	A	A	P	A	A	86	123	6.1	62
82	AMUDHA	51	F	O	A	A	A	A	A	A	A	A	A	A	2	1	OHA	A	A	A	A	A	A	A	A	A	A	A	124	156	6.4	90
83	RAJENDRAN	45	M	O	A	A	A	A	A	A	P	P	A	A	2	5	OHA	P	P	A	P	A	P	A	A	P	A	A	92	143	6.2	43
84	GAYATHRI	58	F	O	A	A	A	P	P	A	A	A	A	A	2	9	OHA	A	A	A	A	A	A	P	A	A	P	A	109	124	6	61
85	THAMBAIYAAH	53	M	O	A	A	A	A	A	A	A	A	A	A	2	9	OHA	A	A	A	A	A	A	A	A	A	A	A	123	145	5.7	89
86	NARASIMAN	59	M	O	A	A	A	A	A	A	P	A	P	A	2	5	OHA	P	A	A	P	A	P	A	A	A	A	A	111	135	6	19
87	VENGAYYA	54	M	O	A	A	A	A	A	A	P	A	P	A	2	8	OHA	P	P	A	P	P	P	A	A	A	P	A	88	133	6.2	66
88	KANAGA	58	F	O	A	A	A	A	A	A	A	A	A	A	2	7	OHA	A	A	A	A	A	P	A	A	A	A	A	97	144	7.2	70
89	AMMAIYAPPAN	51	M	O	A	A	A	A	A	A	P	A	P	A	2	3	OHA	A	A	A	P	A	P	A	A	A	A	A	122	168	7	59
90	JAGADEESAN	27	M	O	A	A	A	A	A	A	A	P	A	A	1	15	INS	P	P	A	A	A	P	A	A	P	A	A	118	154	6.8	44
91	VIMALA	71	F	O	A	A	A	A	A	A	A	A	A	A	2	5	OHA	A	A	A	A	A	A	A	A	A	A	A	129	167	6.2	66
92	ALAGAPPAN	44	M	O	A	A	A	A	A	A	P	A	A	A	2	5	OHA	A	P	P	P	A	P	A	A	A	A	A	112	156	6.1	55
93	MANOHARAN	42	M	O	A	A	A	A	A	A	P	P	P	A	2	2	OHA	A	A	A	P	A	P	A	A	P	A	A	143	178	7.1	40
94	KARTHIKAMMA	78	F	O	A	A	A	A	A	A	A	A	A	A	2	6	OHA	A	A	A	A	A	A	A	A	A	A	A	91	134	5.8	88
95	PALANIVEL	42	M	O	A	A	A	A	A	A	P	A	P	A	2	5	OHA	P	P	P	P	P	A	P	A	A	P	A	124	167	6	34
96	RANGANATHAN	53	M	O	A	A	A	A	A	P	A	A	P	A	2	5	OHA	A	A	A	A	A	A	A	A	A	A	P	114	153	5.9	61
97	KALYANI	59	F	O	A	A	A	A	A	A	P	A	A	A	2	8	OHA	A	A	P	P	A	P	A	A	A	A	A	110	146	6	45
98	RAMAN	34	M	O	A	A	A	A	A	A	A	A	A	A	2	1	OHA	A	A	A	A	A	A	A	A	A	A	A	116	132	5.6	110
99	KANNAN	28	M	O	A	A	A	A	A	A	A	A	A	A	2	1	OHA	P	A	A	A	A	A	A	A	A	A	A	87	116	5.4	102

NO	NAME	AGE	SEX	NEW/OLD	Symptoms														Complications												
					POLYURIA	POLYPHAGIA	POLYDYPSIA	BREATHLESSNESS	CHESTPAIN	OLIGURIA	VISUAL DISTURBANCES	SENSORY MOTOR DISTURBANCES	OBESITY	LEG ULCERS	TYPE	DURATION(YEARS)	TREATMENT(OHA/INSULIN)	SMOKING	ALCOHOLISM	SYSTEMIC HT	RETINOPATHY	CVA	MICROALBUMINURIA	CAD	PERIPHERAL ARTERIAL DISEASE	NEUROPATHY	DYSLIPIDEMIA	ABNORMAL RENAL FUNCTION	FBS	PPBS	HBA1C	SERUM ZINC LEVEL(µg/dl)
100	RANI	54	F	O	A	A	A	A	A	A	P	A	A	A	2	8	OHA	A	A	A	P	P	P	A	A	A	P	A	100	135	6.2	57

CONTROL CHART

Name	Age	Sex	Zinc
palani	43	M	70
subbu	47	M	65
Mani	35	M	81
raja	39	M	88
murugan	46	M	69
guru	45	M	70
suganya	41	F	50
selvi	43	F	53
prabhu	52	M	90
senthil	54	M	51
yogesh	56	M	53
mangamma	67	F	81
gopi	69	M	94
manjula	70	F	91
ravi	34	M	82
ram	39	M	70
rajaram	44	M	67
saravanaa	47	M	68
Ramesh	49	M	86
kala	53	F	74
arasi	55	F	85
vijayakumar	61	M	55
anbu	48	M	57
logesh	49	M	71
selvam	50	M	72
azhageshwari	49	F	99
Rani	50	F	85
babu	51	M	75
jahir	53	M	83
vignesh	31	M	59
suresh	33	M	71
keerthana	32	F	64
subulakshmi	39	F	53
muniammal	37	F	63
lakshmi	42	F	93
kamatchi	44	F	95
krishna	57	M	71
karthick	59	M	74
shanthi	36	F	77
rajn	41	M	79
siva	42	M	80
rajasekar	63	M	80
rani	65	F	89
murugeshwari	58	F	77
indira	60	F	87
rakesh	42	M	89
govind	43	M	65
theivanai	49	F	91
ramasamy	55	M	85
vasanthi	46	F	97

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI -3

Telephone No: 04425305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To
Dr. A. Magesh
PG in MD General Medicine
Madras Medical College, Chennai -3.

Dear Dr. A. Magesh

The Institutional Ethics Committee of Madras Medical College reviewed and discussed your application for approval of the proposal entitled "Serum zinc level in diabetes mellitus & its role in development of complications" No. 21042011.

The following members of Ethics Committee were present in the meeting held on 21.04.2011 conducted at Madras Medical College, Chennai -3.

1.	Prof. S.K. Rajan, MD	-- Chairperson
2.	Prof. V. Kanagasabai MD Dean, Madras Medical College, Chennai-3,	-- Deputy chairman
3.	Prof. A. Sundaram, MD Vice Principal , Madras Medical College, Chennai -3	-- Member Secretary
4.	Prof R. Sathianathan MD	-- Member
5.	Prof R. Nandhini, MD Director, Institute of Pharmacology, MMC, Ch-3	-- Member
6.	Prof. Pregna B. Dolia MD Director , Institute of Biochemistry, MMC, Ch-3	-- Member
7.	Prof. C. Rajendiran .MD Director , Institute of Internal Medicine, MMC, Ch-3	-- Member
8.	Thiru. A. Ulaganathan Administrative Officer, MMC, Chennai -3	-- Layperson
9.	Thiru. S. Govindasamy . BA.BL	-- Lawyer
10.	Tmt. Arnold Soulina	-- Social Scientist

We approve the proposal to be conducted in its presented form.

Sd /. Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, any SAE occurring in the course of the study, any changes in the protocol and patient information / informed consent and asks to be provided a copy of the final report

Member Secretary, Ethics Committee